Angiotensin I Converting Enzyme Activity (ACE1) Assay Kit (Fluorometric)

(Catalog # BN00500; 100 assays, Store kit at -20°C)

I. Introduction:

Angiotensin I converting enzyme (ACE1, EC: 3.4.15.1), a dipeptidyl carboxypeptidase, is part of the renin-angiotensin system (RAS) that controls regulation of blood pressure by cleaving the C-terminal dipeptides of angiotensin I and bradykinin. It is found on the luminal surface of vascular endothelial cells, especially in pulmonary tissues. Elevated levels of ACE1 are found in patients suffering from sarcoidosis, leprosy, hyperthyroidism, acute hepatitis, primary biliary cirrhosis, diabetes mellitus, multiple myeloma, osteoarthritis, amyloidosis, Gaucher's disease, pneumoconiosis, histoplasmosis and miliary tuberculosis. Assay Genies' ACE1 Activity Assay Kit utilizes an active ACE1 to cleave a synthetic *o*-aminobenzoyl peptide (Abz-based peptide) substrate to release a fluorophore. The released Abz can be easily quantified using a fluorescence microplate reader. This assay kit is simple, rapid and can detect ACE activity as low as 10 mU in biological samples.

ACE1 Substrate-Abz $\xrightarrow{\textbf{ACE1}}$ Cleaved ACE1 Substrate + Abz (Fluorescence)
(**Ex./Em. = 330/430 nm**)

II. Applications:

- Detection of ACE1 activity in tissue/cell lysates
- Determination of enzymatic activity of purified ACE1

III. Sample Type:

- Animal tissues: Lung, heart, kidney
- Serum, plasma

IV. Kit Contents:

Components	BN00500-100	Cap Code	Part Number
ACE1 Assay Buffer	20 ml	WM	BN00500-1
ACE1 Lysis Buffer	40 ml	NM	BN00500-2
ACE1 Dilution Buffer	1 ml	Clear	BN00500-3
ACE1 Positive control	5 µl	Green	BN00500-4
ACE1 Substrate	300 µl	Brown	BN00500-5
Abz-Standard (1 mM)	100 µl	Yellow	BN00500-6

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well fluorescence microplate reader.
- BCA Protein Assay Kit - Reducing Agent Compatible (Assay Genie Cat #BN01034, #BN01035 or equivalent).

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the assay**.**

- **ACE1 Lysis Buffer** and **ACE1 Dilution Buffer**: Ready to use. Store at -20ºC. Bring to room temperature before use.
- **ACE1 Lysis Buffer**: Ready to use. Store at -20°C. Thaw before use.
- **ACE1 Positive Control**: Store at -20°C. Avoid multiple freeze/thaw of the enzyme. Use within 6 months.
- **ACE1 Substrate**: Ready to use. Store at -20°C. Thaw before use.
- **Abz Standard**: Ready to use. Store at -20°C

VII. ACE1 Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (~100 mg) or pelleted cells (1-2 x 10^6) with 400 µl ACE1 Lysis Buffer using dounce homogenizer, keep on ice for 10 min. Vortex gently for 10 s., and keep on ice for another 5 min. Centrifuge the homogenate at 16,000 x g, 4°C for 10 min. Discard the pellet.

   Protein concentration measurement: Transfer the clarified supernatant to a clean pre-chilled tube and keep on ice. Measure the amount of protein in the lysate or purified enzyme using BCA Protein Assay Kit, Reducing Agent Compatible (Assay Genie product BN01034, BN01035 or equivalent).

   Experimental procedure: Add 1-10 µl of lysate (maximum up to 2 µg of protein) into desired well(s) in a 96-well plate. If necessary, dilute the lysate or enzyme with ACE1 Lysis buffer. For Background Control, add lysis buffer. For Positive Control, add 95 µl of ACE1 Dilution Buffer to the ACE1 Positive control vial and use 10 µl of the diluted ACE1 Solution into desired well(s). Adjust the volume of Samples, Background Control & Positive Control to 50 µl /well with ACE1 Assay Buffer.

   Notes:

   a. We recommend using the tissue/cell homogenate immediately to measure the ACE1 activity. If desired, snap freeze the lysate and store at -80°C. Unused diluted ACE1 Positive Control can be stored at -20°C in small aliquots.

   b. Tissue or cell lysates of more than 2 µg of total protein/well might suppress the enzymatic activity of ACE1 with the provided substrate. In that case, dilute the sample with ACE1 Lysis Buffer and use 3-5 different amounts of the diluted lysate per well.

c. Plasma or serum samples can be used directly in the assay. The volume used per well will need to be optimized by the user.

2. **Abz-Standard Curve Preparation:** Prepare 100 µM solution of Abz-Standard by diluting 50 µl of 1 mM Abz-Standard with 450 µl of ACE1 Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µl of 100 µM Abz-Standard into a series of wells in a 96-well plate and adjust the final volume to100 µl/well with ACE1 Assay Buffer to generate 0, 200, 400, 600, 800 and 1000 pmol/well of Abz-Standard respectively. Mix well and measure the fluorescence (Ex/Em = 330/430 nm) in an end point mode.
3. **ACE1 Substrate Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix:

   47 µl ACE1 Assay Buffer
   3 µl ACE1 Substrate

   Mix & add 50 µl of ACE1 Substrate Mix into each sample, and Positive Control well. Mix well.

   Note: Don't add Substrate Mix to the Standard wells.
4. **Measurement:** Measure fluorescence (Ex/Em = 330/430 nm) in a kinetic mode for 1-2 hr at 37°C. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2). Calculate ΔRFU/ΔT.
5. **Calculation**: Subtract 0 Standard reading from all readings. Plot the Abz-Standard Curve and obtain the slope of the curve (ΔRFU/pmol). If sample background control reading is significant then subtract the background control reading from sample reading.

$$\textbf{Sample ACE1 Activity} = \textbf{B X D}/(\textbf{ΔT} * \textbf{mg of protein}) = \textbf{pmol/min/mg} = \textbf{mU/mg}$$

Where:

B = Abz in sample based on Std. curve slope (pmol)
ΔT = reaction time (min.)
V = sample volume added into the reaction well (ml)
D = sample dilution factor (D=1 when samples are undiluted

Unit Definition: One unit of ACE1 activity is the amount of enzyme that catalyzes the release of 1 nmol of Abz per min from the substrate under the assay conditions at 37°C.

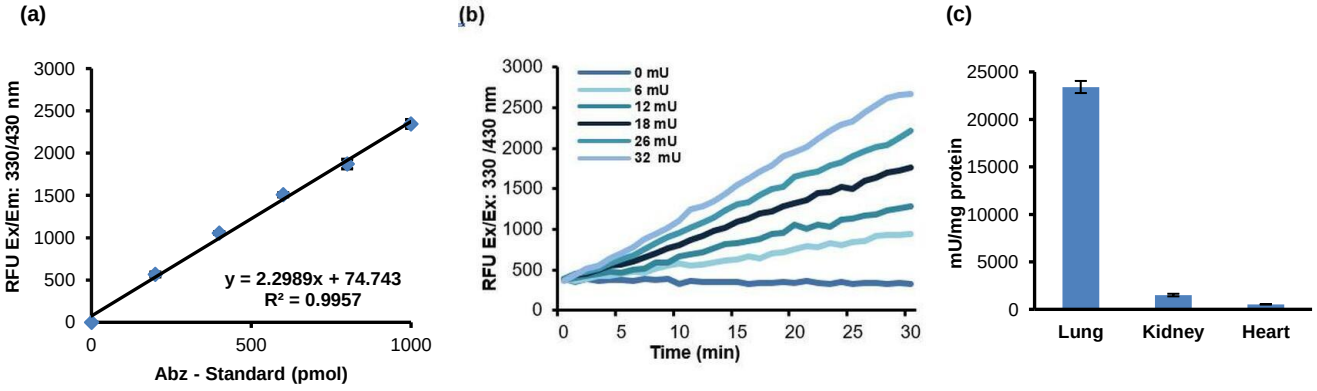

Figure: (a) Abz-Standard Curve (0-1000 pmol), error bars indicate SD (n=3). (b) Kinetic activity curves using different amounts of ACE1 Positive Control in the assay. (c) ACE1 activity was measured for different types of rat tissue samples (lung, heart and kidney; 0.6 µg each). Assays were performed following the kit protocol.

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